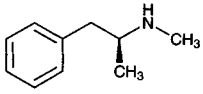
KEY WORDS
chiral

REFERENCE
Kristensen,K.; Angelo,H.R. A stereoselective HPLC method for the determination of methadone and is main metabolite in urine (using an AGP column) (Abstract 39), *Ther.Drug Monit.*, **1995**, *17*, 393–393.

Methamphetamine



Molecular formula: $C_{10}H_{15}N$
Molecular weight: 149.24
CAS Registry No.: 537-46-2
Merck Index: 6015
Lednicer No.: 1 37

SAMPLE
Matrix: blood
Sample preparation: Inject a 5 μL aliquot of serum directly.

HPLC VARIABLES
Column: 100 × 4.6 5-10 μm Silicalite (by sieving Silicalite, 3M Co.(?))
Mobile phase: MeNC:20 mM pH 6.9 phosphate buffer 10:90
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 3.79

OTHER SUBSTANCES
Extracted: ethosuximide, sulfamethoxazole, primidone

KEY WORDS
serum

REFERENCE
Ambrose,D.L.; Fntz,J.S. High-performance liquid chromatographic determination of drugs and metabolites in human serum and urine using direct injection and a unique molecular sieve, *J.Chromatogr.B*, **1998**, *709*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 100 mM NaOH + 3 mL n-hexane, shake for 20 min, centrifuge for 10 min. Remove 2 mL of the organic layer and evaporate it to dryness using a vacuum centrifuge, reconstitute the residue in 500 μL 100 μg/mL (S)-(+)-benoxaprofen chloride in dried dichloromethane, let stand at room temperature for 30 min, inject a 10 μL aliquot. (Synthesis of benoxaprofen chloride is as follows. Dissolve 600 mg benoxaprofen in 50 mL toluene, slowly add 5 mL freshly-distilled thionyl chloride, reflux for 30 min, evaporate to dryness, recrystallize benoxaprofen chloride from dichloromethane.)

HPLC VARIABLES
Column: 250 × 4.6 7 μm Zorbax-Sil
Mobile phase: Cyclohexane:dichloromethane:THF 50:10:10
Flow rate: 1
Injection volume: 10
Detector: F ex 312 em 365

CHROMATOGRAM
Retention time: 10.5 (R-(-)), 11.5 (S-(+))

OTHER SUBSTANCES
Extracted: amphetamine
Interfering: tranylcypromine

KEY WORDS
plasma; derivatization; normal phase; chiral

REFERENCE
Weber,H.; Spahn,H.; Mutschler,E.; Möhrke,W. Activated α-alkyl-α-arylacetic acid enantiomers for stereoselective thin-layer chromatographic and high-performance liquid chromatographic determination of chiral amines, *J.Chromatogr.*, **1984**, *307*, 145–153.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 1 mL acetone, vortex for 2 min, centrifuge at 2000 g for 10 min. Remove a 500 μL aliquot of the supernatant and add it to 500 μL reagent, add 20 μL 0.5% triethylamine in MeOH, vortex, heat at 80° for 30 min, cool to room temperature, inject a 20 μL aliquot. (Prepare reagent by mixing 2 mL 2.5 mM N-(4-aminobutyl)-N-ethylisoluminol in MeOH with 2 mL 2.5 mM N,N'-disuccinimidyl carbonate in MeCN, let stand for 2 h.)

HPLC VARIABLES
Guard column: 30 × 4.6 TSKm Guardgel ODS-80TM (Toyo Soda)
Column: 150 × 6 5 μm Shimpack CLC C18 (Shimadzu)
Mobile phase: MeOH:water 54:46 containing 30 mM sodium 1-octanesulfonate
Flow rate: 1
Injection volume: 20
Detector: Chemiluminescence following post-column reaction. The column effluent mixed with 15 mM potassium ferricyanide in 2.5 M NaOH pumped at 1 mL/min and this mixture flowed through a 200 mm × 0.5 mm ID stainless steel coil. The effluent from this coil mixed with 300 mM hydrogen peroxide containing 10 mM β-cyclodextrin pumped at 1 mL/min and this mixture flowed through a 100 mm × 0.5 mm ID stainless steel coil to the detector.

CHROMATOGRAM
Retention time: 30
Limit of detection: 20 pM

KEY WORDS
derivatization; serum

REFERENCE
Nakashima,K.; Suetsugu,K.; Akiyama,S.; Yoshida,M. High-performance liquid chromatography-chemiluminescence determination of methamphetamine in human serum using N-(4-aminobutyl)-N-ethylisoluminol as a chemiluminogen, *J.Chromatogr.*, **1990**, *530*, 154–159.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 50 μL 100 ng/mL aniline sulfate in water + 200 μL 20 mM pH 10.6 carbonate buffer + 2 mL ethyl acetate, shake for 15 min, centrifuge at 1200 g for 5 min. Remove the organic layer and add it to 200 μL 50 mM HCl, shake for 15 min, centrifuge at 1200 g for 5 min. Remove the aqueous layer and add it to 40 μL 250 mM NaOH, add 50 μL 330 mM pH 7.8 phosphate buffer, add 250 μL MeCN, add 25 μL 1 mM (-)-1-(9-fluorenyl)ethyl chloroformate in acetone, let stand overnight at room temperature, add 30 μL 100 mM glycine in water, add 750 μL n-pentane, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness under vacuum, reconstitute the residue in MeCN: water 50:50, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: Direct-Connect column prefilter (Alltech)

Column: 150 × 4.6 3 μm Adsorbosphere HS C18 (Alltech)
Mobile phase: MeCN:THF:20 mM pH 3.6 acetate buffer 39:15:46
Flow rate: 1
Injection volume: 100
Detector: F ex 265 em 330

CHROMATOGRAM
Retention time: 27.7 (R), 29.0 (S)
Internal standard: aniline (21.0)
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
serum; rat; chiral; derivatization

REFERENCE
Hutchaleelaha,A.; Walters,A.; Chow,H.-H.; Mayersohn,M. Sensitive enantiomer-specific high-performance liquid chromatographic analysis of methamphetamine and amphetamine from serum using precolumn fluorescent derivatization, *J.Chromatogr.B*, **1994**, *658*, 103–112.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 1 mL 500 mM pH 11 borate buffer, mix, add 2.5 mL diethyl ether, vortex for 5 min, centrifuge at 1200 g for 5 min, remove organic layer, repeat extraction. Combine the organic layers and add them to 200 μL 100 mM HCl, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 150 μL 1 M pH 8 borate buffer and 100 μL 4 mM 9-fluorenylmethyl chloroformate in MeCN, shake, allow to react at 50° for 5 min, add 20 μL 20 mM proline in water, allow to react at 50° for 2 min, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: MeCN:50 mM pH 6.0 sodium phosphate buffer 50:50
Flow rate: 1
Injection volume: 200
Detector: F ex 260 em 315

CHROMATOGRAM
Retention time: 15
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine, desmethyldeprenyl

KEY WORDS
plasma

REFERENCE
La Croix,R.; Pianezzola,E.; Strolin Benedetti,M. Sensitive high-performance liquid chromatographic method for the determination of the three main metabolites of selegiline (L-deprenyl) in human plasma, *J.Chromatogr.B*, **1994**, *656*, 251–258.

SAMPLE
Matrix: blood, urine
Sample preparation: Adjust pH of 10 mL plasma or 20 mL urine to 11.4 with 5 M NaOH, add to a column containing 1.5 g Amberlite XAD-2, wash with 10 mL water, elute with 20 (plasma) or 40 (urine) mL chloroform:isopropanol 75:25, add 100 μL 6 M HCl in EtOH to the eluate, evaporate to dryness under reduced pressure, reconstitute with 1 mL 8% sodium bicarbonate,

add 1 mL 0.5% sodium 1,2-naphthoquinone-4-sulfonate, heat at 70° for 20 min, add an equal volume of chloroform, vortex for 1 min, inject a 50 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 150 × 5 Partisil 5
Mobile phase: Hexane:chloroform:ethyl acetate:EtOH 50:25:35:1
Column temperature: 20
Flow rate: 2.5
Injection volume: 50
Detector: UV 248

CHROMATOGRAM
Retention time: 3
Internal standard: phenylethylamine (6)
Limit of detection: 2 ng

OTHER SUBSTANCES
Extracted: amphetamine, hydroxyamphetamine, norephedrine

KEY WORDS
derivatization; plasma; normal phase; SPE; comparison with other derivatization reagents and with ion-pair chromatography

REFERENCE
Farrell,B.M.; Jefferies,T.M. An investigation of high-performance liquid chromatographic methods for the analysis of amphetamines, *J.Chromatogr.*, **1983**, *272*, 111–128.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 206.4

CHROMATOGRAM
Retention time: 8.433

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Mix a 1 mg/mL solution in 1 M sodium carbonate with 2 mL 5 mg/mL 8-quinolinesulfonyl chloride in acetone, heat at 65° for 20 min, cool, extract twice with 30 mL portions of chloroform. Combine the extracts and dry them over anhydrous magnesium sulfate, evaporate to dryness under a stream of air, reconstitute, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 Co:Pell ODS
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 40:59:1
Flow rate: 1.5
Detector: UV 254, UV 280

CHROMATOGRAM
Retention time: 25

OTHER SUBSTANCES
Simultaneous: amphetamine, ephedrine, phenmetrazine, phentermine, phenylpropanolamine, pseudoephedrine

KEY WORDS
derivatization

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R. Liquid chromatographic determination of primary and secondary amines as 8-quinolinesulfonyl chloride derivatives, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 687–691.

SAMPLE
Matrix: bulk
Sample preparation: Prepare an aqueous solution. Adjust pH of 500 μL aqueous solution to 12 with 1 M NaOH, add 200 μL (+)-1-(9-fluorenyl)ethyl chloroformate:dichloromethane 1:100, let stand for 20 min, add 500 μL dichloromethane, shake for 30 min. Remove the organic phase and wash it twice with 500 μL aliquots of water, evaporate the organic phase to dryness under a stream of nitrogen, reconstitute the residue in 3 mL MeOH, filter (0.45 μm), inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 3.9 5 μm 5C18-AR (Waters)
Mobile phase: MeCN:50 mM pH 6.0 phosphate buffer 65:35
Flow rate: 1
Injection volume: 25
Detector: F ex 295 em 315

CHROMATOGRAM
Retention time: 44 ((S)-(+)), 47 ((R)-(-))
Limit of quantitation: 16.7 ng/mL

OTHER SUBSTANCES
Extracted: ephedrine, pseudoephedrine

KEY WORDS
derivatization; chiral

REFERENCE
Chen,Y.-P.; Hsu,M.-C.; Chien,C.S. Analysis of forensic samples using precolumn derivatization with (+)-1-(9-fluorenyl)ethyl chloroformate and liquid chromatography with fluorimetric detection, *J.Chromatogr.A*, **1994**, *672*, 135–140.

SAMPLE
Matrix: bulk

Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES

Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: k' 14.33, k' 15.90 (enantiomers)

OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, atenolol, carazolol, carvedilol, formoterol, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, pindolol, propranolol, xamoterol

KEY WORDS

derivatization; chiral

REFERENCE

Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE

Matrix: formulations
Sample preparation: Powder tablet and add 50 mg to 50 mL MeCN:20 mM pH 3.8 phosphate buffer 3:97, sonicate for 5 min, filter (0.5 μm), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: Supelguard pre-column containing 5 μm Suplex pKb100 (Supelco)
Column: 150 × 4.6 5 μm Suplex pKb100 (Supelco)
Mobile phase: Gradient. MeCN:20 mM pH 3.8 phosphate buffer at 3:97 for 3 min, to 15:85 over 5 min, stay at 15:85 for 4 min, re-equilibrate for 8 min.
Flow rate: 1.5
Injection volume: 20
Detector: UV 220 for 5 min then UV 280

CHROMATOGRAM

Retention time: 4.3
Limit of quantitation: 10 μg/mL

OTHER SUBSTANCES

Simultaneous: ephedrine, amphetamine, caffeine, 3,4-methylenedioxyamphetamine, N-methyl-3,4-methylenedioxyamphetamine, N-ethyl-3,4-methylenedioxyamphetamine

KEY WORDS
tablets

REFERENCE
Longo,M.; Martines,C.; Rolandi,L.; Cavallaro,A. Simple and fast determination of some phenethylamines in illicit tablets by base-activated reversed phase HPLC, *J.Liq.Chromatogr.*, **1994**, *17*, 649–658.

SAMPLE
Matrix: solutions
Sample preparation: Mix 1 mL 20-300 μg/mL amine solution in water with 2 mL 50 mg/mL 4-nitrobenzoyl chloride in THF (freshly prepared) and 1 mL 1 M NaOH, heat at 65° for 1 h, cool, adjust pH to 12 with 1 M NaOH, extract with two 10 mL portions of chloroform. Combine the extracts and wash them with two 20 mL portions of 10% potassium carbonate, wash with water, dry over anhydrous magnesium sulfate. Evaporate to dryness under a stream of air, reconstitute the residue in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water 35:65
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: amphetamine, benzylamine, α-methylbenzylamine, n-propylamphetamine

KEY WORDS
derivatization

REFERENCE
Clark,R.C.; Teague,J.D.; Wells,M.M.; Ellis,J.H. Gas and high-pressure liquid chromatographic properties of some 4-nitrobenzamides of amphetamines and related arylalkylamines, *Anal.Chem.*, **1977**, *49*, 912–915.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 4.05

OTHER SUBSTANCES
Simultaneous: dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine, pemoline, benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, mephentermine, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodine

Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine
Interfering: dimethylamphetamine, mescaline, norpethidine, hydrocodone

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions
Sample preparation: Mix 1 mL of an aqueous solution with 1 mL 100 mM nickel sulfate in water, 1 mL 20% aqueous ammonia, and 5 mL chloroform:carbon disulfide 98:2, shake vigorously for 1 min, wash the organic layer with three 2 mL portions of water, filter (phase-separation paper). Evaporate the filtrate to dryness under a stream of nitrogen, reconstitute with 1 mL mobile phase, inject a 10 μL aliquot. (Copper may also be used with electrochemical detection or UV detection at 270 nm.)

HPLC VARIABLES

Guard column: 30 × 4 40 μm LiChrosorb RP-18
Column: 250 × 4 7 μm LiChrosorb RP-18
Mobile phase: MeOH:20 mM pH 5.8 sodium acetate buffer 80:20 containing 5 mM lithium perchlorate
Flow rate: 1.5
Injection volume: 10
Detector: UV 325, E, Merck-Clevenot E 230, Model LCC 231 thin-layer electrolytic cell with a glassy carbon electrode at +0.7 V, standard calomel reference electrode

CHROMATOGRAM

Retention time: 8.49
Limit of detection: 1 fmole (E), 1 nmole (UV)

OTHER SUBSTANCES

Simultaneous: ephedrine
Also analyzed: acebutolol, alprenolol, flecainide, propranolol

KEY WORDS

derivatization; complexation

REFERENCE

Leroy,P.; Nicolas,A. Determination of secondary amino drugs as their metal dithiocarbamate complexes by reversed-phase high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1984**, *317*, 513–521.

SAMPLE

Matrix: solutions
Sample preparation: 2 mL THF + 1 mL 33.5 mM reagent in THF (freshly prepared) + 1 mL 1 mg/mL amphetamine in water + 700 μL 10% sodium bicarbonate in water, heat at 65° for 1 h, cool, extract three times with 10 mL aliquots of chloroform. Combine the extracts and wash them with 10 mL water, dry over anhydrous magnesium sulfate, evaporate to dryness, reconstitute with 2.5 mL mobile phase, inject a 5 μL aliquot. (Prepare reagent (1-[(4-nitrophenyl)sulfonyl]prolyl chloride) as follows. Mix 40-45 mmoles L-(-)-proline, 40 mL THF, and 200 mL 10% potassium carbonate, add 37-43 mmoles 4-nitrobenzenesulfonyl chloride in 40 mL THF dropwise, heat at 50° and maintain at pH 8 or above for 3 h, cool, acidify to pH 2, extract with chloroform. Extract the organic layers with potassium carbonate in water. Acidify the aqueous layer and extract it with chloroform. Dry the chloroform layer and evaporate it to dryness, recrystallize the resulting 1-[(4-nitrophenyl)sulfonyl]proline from petroleum ether and benzene (Caution! Benzene is a carcinogen!). Stir 15 mmoles 1-[(4-nitrophenyl)sulfonyl]proline in 100 mL benzene and add 75 mmoles thionyl chloride in 50 mL benzene dropwise, heat at 35-40° until the reaction is complete (about 48 h; monitor by IR), evaporate to dryness, recrystallize from n-heptane to give 1-[(4-nitrophenyl)sulfonyl]prolyl chloride (Anal.Chem. 1984, 56, 958) (mp 110-110.5°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Zorbax ODS

Mobile phase: MeOH:water 60:40
Flow rate: 1.5
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 12 (S), 14 (R)

KEY WORDS
derivatization; chiral

REFERENCE
Barksdale,J.M.; Clark,C.R. Liquid chromatographic determination of the enantiomeric composition of amphetamine and related drugs by diastereomeric derivatization, *J.Chromatogr.Sci.*, **1985**, *23*, 176–180.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldi-

amine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 15:1.5:0.5:83
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 13

OTHER SUBSTANCES

Simultaneous: phenylpropanolamine, ephedrine, hydroxyamphetamine, amphetamine, phentermine, mephentermine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions
Sample preparation: 100 μL 100 μM Solution + 300 μL buffer + 500 μL 1 mM dansyl chloride in acetone, mix, heat at 45° in the dark for 1 h, dilute with MeCN:water 50:50, inject a 20 μL aliquot. (Prepare buffer by adjusting 10 mM sodium bicarbonate to pH 9.0 with NaOH.)

HPLC VARIABLES

Guard column: 30 × 4.6 Spheri-5 RP-18
Column: 250 × 4.6 Inertsil ODS-2
Mobile phase: MeCN:water 70:30 containing 1 mM imidazole, pH adjusted to 7.0 with nitric acid
Flow rate: 1
Injection volume: 20
Detector: Chemiluminescence following post-column reaction. The column effluent mixed with the reagent pumped at 1 mL/min and the mixture flowed through a 300 mm × 0.25 mm ID coil to the detector. (Prepare the reagent by dissolving 112 mg bis(2,4,6-trichlorophenyl) oxalate in 500 mL MeCN, add 8.6 mL 30% hydrogen peroxide, sonicate.), F ex 343 em 530

CHROMATOGRAM

Retention time: 18.5
Limit of detection: 4 fmole (chemiluminescence), 50 fmole (F)

OTHER SUBSTANCES

Simultaneous: benzylamine, ephedrine, N-isopropylbenzylamine, N-methylphenethylamine, phenylbutylamine, phenylethylamine, phenylpropanolamine, phenylpropylamine

KEY WORDS

derivatization; post-column reaction; comparison with other derivatization reagents

REFERENCE

Hayakawa,K.; Hasegawa,K.; Imaizumi,N.; Wong,O.S.; Miyazaki,M. Determination of amphetamine-related compounds by high-performance liquid chromatography with chemiluminescence and fluorescence detections, *J.Chromatogr.*, **1989**, *464*, 343–352.

SAMPLE

Matrix: solutions

Sample preparation: 100 μL 100 μM Solution + 400 μL buffer + 500 μL 80 mM 4-fluoro-7-nitrobenzoxadiazole in EtOH, mix, heat at 60° in the dark for 1 min, dilute with MeCN:water 50:50, inject a 20 μL aliquot. (Prepare buffer by adjusting 100 mM boric acid to pH 8.0 with NaOH.)

HPLC VARIABLES

Guard column: 30 × 4.6 Spheri-5 RP-18

Column: 250 × 4.6 Inertsil ODS-2

Mobile phase: MeCN:water 60:40 containing 1 mM imidazole, pH adjusted to 7.0 with nitric acid

Flow rate: 1

Injection volume: 20

Detector: Chemiluminescence following post-column reaction. The column effluent mixed with the reagent pumped at 1 mL/min and the mixture flowed through a 300 mm × 0.25 mm ID coil to the detector. (Prepare the reagent by dissolving 112 mg bis(2,4,6-trichlorophenyl) oxalate in 500 mL MeCN, add 8.6 mL 30% hydrogen peroxide, sonicate.), F ex 470 em 530

CHROMATOGRAM

Retention time: 17

Limit of detection: 20 nmole (chemiluminescence), 30 nmole (F)

OTHER SUBSTANCES

Simultaneous: benzylamine, ephedrine, N-isopropylbenzylamine, N-methylphenethylamine, phenylbutylamine, phenylethylamine, phenylpropanolamine, phenylpropylamine

KEY WORDS

derivatization; post-column reaction; comparison with other derivatization reagents

REFERENCE

Hayakawa,K.; Hasegawa,K.; Imaizumi,N.; Wong,O.S.; Miyazaki,M. Determination of amphetamine-related compounds by high-performance liquid chromatography with chemiluminescence and fluorescence detections, *J.Chromatogr.*, **1989**, *464*, 343–352.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a 500 μg/mL solution in MeOH:water 50:50, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Zorbax C8

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L MeCN:water 20:80. A:B from 100:0 to 0:100 over 30 min. (Purify triethylamine as follows. Wash neutral alumina (Merck) 3 times with 2 bed volumes of pentane, 3 times with 2 bed volumes of dichloromethane, and 3 times with 2 bed volumes of MeOH, allow solvent to evaporate in a fume hood overnight, heat alumina at 130° for 2 h. Prepare a 14 cm column of the washed alumina in a 290 × 22 tube, pass through a head volume of MeOH, pass through triethylamine. When triethylamine starts to elute discard the first 20 mL, use the next 20 mL, discard the column.)

Flow rate: 2

Injection volume: 5

Detector: UV 210

CHROMATOGRAM

Retention time: 10

OTHER SUBSTANCES
Simultaneous: acetophenone, amphetamine, desipramine, ethylmorphine, imipramine, mefenamic acid, morphine, phenylbutazone, salicylic acid

KEY WORDS
also details of isocratic elution

REFERENCE
Hill,D.W. Evaluation of alkyl bonded silica and solvent phase modifiers for the efficient elution of basic drugs on HPLC, *J.Liq.Chromatogr.*, **1990**, *13*, 3147–3175.

SAMPLE
Matrix: solutions
Sample preparation: Mix 50 μL of a 200 ppm solution in MeCN:500 mM pH 9.0 borate buffer 50:50 with 25 mg reagent, after 1 min elute with 1 mL hexane:THF 75:25, inject a 5 μL aliquot. (Reagent was dinitrophenyl carbamate benzotriazole polymeric reagent, synthesized as follows. (Caution! Chloroform, dichloromethane, dioxane, and hydrazine are carcinogenic in experimental animals! DMF may be carcinogenic! 3,5-dinitrobenzoyl chloride and aluminum chloride are corrosive! Nitrobenzene is toxic!) 10 g Dried macroporous polystyrene (Xe-305, Rohm and Haas) + 10 g 3-nitro-4-chlorobenzyl alcohol + 10 g anhydrous aluminum chloride + 50 mL nitrobenzene, heat at 65-70° for 3 days, cool, filter, wash polymer with three 50 mL portions of 1 M HCl in dioxane, with three 50 mL portions of DMF,with three 50 mL portions of MeOH, and with three 50 mL portions of dichloromethane, dry under vacuum at 100°. Reflux 19 g of this polymer in 60 mL hydrazine hydrate:ethylene glycol monoethyl ether 40:60 for 20 h, cool to room temperature, filter off the polymer and wash it thoroughly with water. Suspend the polymer in 100 mL concentrated HCl:dioxane 50:50, reflux for 20 h, filter the polymer and wash it with five 100 mL portions of water, with three 100 mL portions of MeOH, and with three 50 mL portions of ether, dry under vacuum at 80°. Functionalization was 1.17 mmoles/g (Eur.J.Biochem. 1975, 59, 55). Dissolve 3,5-dinitrobenzoyl chloride in the minimum amount of glacial acetic acid, add an equimolar amount of sodium azide, stir for 30 min, dilute with water, filter to obtain 3,5-dinitrobenzoyl azide (Caution! Azides are toxic and potentially explosive!) (J. Liq. Chromatogr. 1986,9, 443). Heat 71 mg 3,5-dinitrobenzoyl azide in 15 mL toluene (dried over calcium hydride) at ??? for 30 min, cool using an ice bath, add 200 mg polymer, allow to warm to room temperature with stirring for 1 h, filter, wash the polymer with four 10 mL portions of warm (40°) dichloromethane, dry under high vacuum for 1 h.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm LC-(R)-naphthylurea (Supelco)
Mobile phase: Hexane:EtOH:MeCN 93:7:0.5
Flow rate: 2
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 8.7, 10.7 (enantiomers)

OTHER SUBSTANCES
Simultaneous: amphetamine

KEY WORDS
derivatization; chiral

REFERENCE
Bourque,A.J.; Krull,I.S. Immobilized isocyanates for derivatization of amines for chiral recognition in liquid chromatography with UV detection, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 495–503.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 30 × 2.1 Spheri-5 RP-8

Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: 7

OTHER SUBSTANCES
Simultaneous: diethylpropion, phenylpropanolamine, ephedrine, amphetamine, phentermine, fenfluramine
Also analyzed: amitriptyline, chlordiazepoxide, chlorpromazine, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, doxepin, flurazepam, imipramine, mesoridazine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

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REFERENCE
Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepi-

nephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 5 μm Chiradex (immobilized β-cyclodextrin) (Merck)
Mobile phase: MeOH:100 mM pH 7 ammonium acetate 5:95
Column temperature: 20
Flow rate: 0.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 30.28 (S(+)), 31.93 (R(-))

KEY WORDS

chiral

REFERENCE

Rizzi,A.M.; Hirz,R.; Cladrowa-Runge,S.; Jonsson,H. Enantiomeric separation of amphetamine, methamphetamine and ring substituted amphetamines by means of a β-cyclodextrin-chiral stationary phase, *Chromatographia*, **1994**, *39*, 131–137.

SAMPLE

Matrix: solutions
Sample preparation: 1 mL Solution + 500 μL 0.5% sodium 1,2-naphthoquinone-4-sulfonate in water + 500 μL buffer, let stand for 10 min, extract three times with 2 mL aliquots of n-hexane: ethyl acetate 50:50. Combine the organic layers and evaporate them to dryness at 80°, reconstitute with 2 mL MeCN:water 50:50, inject a 50 μL aliquot. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES

Column: 250 × 4 5 μm Hypersil ODS C18
Mobile phase: Gradient. MeCN:0.5% propylamine hydrochloride in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30 for 4.5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM

Retention time: 7

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
derivatization

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. On-line derivatization into precolumns for the determination of drugs by liquid chromatography and column switching: Determination of amphetamines in urine, *Anal.Chem.*, **1996**, *68*, 734–739.

SAMPLE
Matrix: solutions
Sample preparation: 500 μL Solution + 250 μL buffer + 250 μL 20 mM 9-fluorenylmethyl chloroformate in MeCN, mix, add 1 mL MeCN, inject a 50 μL aliquot. (Prepare buffer by adjusting the pH of 4% sodium bicarbonate to 10 with 10% NaOH.)

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100 RP 18
Mobile phase: Gradient. MeCN:water from 40:60 to 50:50 over 2.5 min, to 70:30 over 2.5 min, to 100:0 over 5 min.
Flow rate: 1.5
Injection volume: 50
Detector: F ex 264 em 313

CHROMATOGRAM
Retention time: 8.8

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
derivatization

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. On-line derivatization into precolumns for the determination of drugs by liquid chromatography and column switching: Determination of amphetamines in urine, *Anal.Chem.*, **1996**, *68*, 734–739.

SAMPLE
Matrix: urine
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH, 3 mL MeCN: 10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, and 5 mL water. 5 mL Urine + 5 mL 500 mM ammonium acetate, adjusted to pH 9.5 with ammonia, mix, add to the SPE cartridge, wash with 20 mL 5 mM pH 9.5 ammonium acetate, wash with 0.5 mL water. Elute with 2 mL MeCN:10 mM ammonium acetate 40:60 adjusted to pH 3 with acetic acid, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 4.6 L-column ODS (Chemical Inspection & Testing Institute, Tokyo)
Mobile phase: Gradient. MeCN:100 mM ammonium acetate 0:100 for 1 min, to 40:60 over 20 min.
Flow rate: 1
Injection volume: 50
Detector: UV 210; MS Shimadzu model QP-1100EX thermospray, vaporizer temperature from 170 to 150° over 20 min. SIM, m/z 150

CHROMATOGRAM
Retention time: 15
Limit of detection: 2–40 ng/mL

OTHER SUBSTANCES
Extracted: 6-acetylmorphine, amphetamine, benzoylecgonine, cocaine, ephedrine, methylephedrine, morphine, morphine-3-glucuronide, morphine-6-glucuronide

KEY WORDS
SPE

REFERENCE
Tatsuno,M.; Nishikawa,M.; Katagi,M.; Tsuchihashi,H. Simultaneous determination of illicit drugs in human urine by liquid chromatography-mass spectrometry, *J.Anal.Toxicol.*, **1996**, *20*, 281–286.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + N-ethylnordiazepam + chlorpheniramine + 100 μL buffer, centrifuge at 11000 g for 30 s, inject a 500 μL aliquot onto column A with mobile phase A, after 0.6 min backflush column A with mobile phase A to waste for 1.6 min, elute column A with 250 μL mobile phase B, with 200 μL mobile phase C, and with 1.15 mL mobile phase D. Elute column A to waste until drugs start to emerge then elute onto column B. Elute column B to waste until drugs started to emerge, then elute onto column C. When all the drugs have emerged from column B remove it from the circuit, elute column C with mobile phase D, monitor the effluent from column C. Flush column A with 7 mL mobile phase E, with mobile phase D, and mobile phase A. Flush column B with 5 mL mobile phase E then with mobile phase D. (Buffer was 6 M ammonium acetate adjusted to pH 8.0 with 2 M KOH.)

HPLC VARIABLES
Column: A 10 × 2.1 12-20 μm PRP-1 spherical poly(styrene-divinylbenzene) (Hamilton); B 10 × 3.2 11 μm Aminex A-28 (Bio-Rad); C 25 × 3.2 5 μm C8 (Phenomenex) + 150 × 4.6 5 μm silica (Macherey-Nagel)
Mobile phase: A 0.1% pH 8.0 potassium borate buffer; B 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid; C MeCN:buffer 40:60 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); D MeCN:buffer 33:67 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.); E MeCN:buffer 70:30 (Buffer was 6 mM KH_2PO_4 containing 5 mM tetramethylammonium hydroxide, and 2 mM dimethyloctylamine, pH adjusted to 6.50 with phosphoric acid.)
Column temperature: ambient (column A), 40 (columns B and C)
Flow rate: A 5; B-E 1
Injection volume: 500
Detector: UV 210, UV 235

CHROMATOGRAM
Retention time: k' 3.1
Internal standard: N-ethylnordiazepam (k' 2.1), chlorpheniramine (k' 5.9)
Limit of detection: 300 ng/mL

OTHER SUBSTANCES
Extracted: methadone, imipramine, flurazepam, amitriptyline, morphine, codeine, hydromorphone, hydrocodone, caffeine, cotinine, benzoylecgonine, secobarbital, oxazepam, phenobarbital, nordiazepam, diazepam, phenylpropanolamine, phentermine, amphetamine, phenmetrazine, lidocaine, ephedrine
Interfering: pentazocine, desipramine, nortriptyline, diphenhydramine

KEY WORDS
column-switching

REFERENCE
Binder,S.R.; Regalia,M.; Biaggi-McEachern,M.; Mazhar,M. Automated liquid chromatographic analysis of drugs in urine by on-line sample cleanup and isocratic multi-column separation, *J.Chromatogr.*, **1989**, *473*, 325–341.

SAMPLE
Matrix: urine
Sample preparation: 500 μL Urine + 100 μL 25 μg/mL N-n-propylaniline + 6 mL pH 10.0 carbonate buffer + 15 mL water, add mixture to an Extrelut SPE cartridge, let stand for 20 min, elute with 40 mL hexane:ethyl acetate 90:10. Add the eluate to 3 mL 100 mM sulfuric acid and 500 mg NaCl, stir for 20 min, centrifuge at 1000 g for 5 min. Remove the lower layer and add it to 3 mL 2.5 M NaOH and 20 μL benzoyl chloride, stir vigorously for 30 min. Extract the mixture with 1.5 mL chloroform. Wash the chloroform layer twice with 5 mL water and evaporate it to dryness at 40°, reconstitute the residue in 200 μL hexane:isopropanol 90:10, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralcel OB + 250 × 4.6 Chiralcel OJ
Mobile phase: Hexane:isopropanol 90:10
Column temperature: 48
Flow rate: 1-1.4
Detector: UV 220

CHROMATOGRAM
Retention time: 13 (d), 15 (l)
Internal standard: N-n-propylaniline (11)
Limit of detection: 25 ng

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
rat; SPE; derivatization; chiral

REFERENCE
Nagai,T.; Kamiyama,S. Assay of the optical isomers of methamphetamine and amphetamine in rat urine using high-performance liquid chromatography with chiral cellulose-based columns, *J.Chromatogr.*, **1990**, *525*, 203–209.

SAMPLE
Matrix: urine
Sample preparation: 200-500 μL Rat urine + 200-500 μL pH 3.8 acetate buffer + 25 μL 40 μg/mL β-glucuronidase and 20 μg/mL arylsulfatase (Merck), heat at 37° for 24 h, add 100 μL 25 μg/mL 3-methoxytyramine in water, add 100 μL water, adjust pH to 9.0 with 1.9 M sodium carbonate, add to an Extrelut SPE cartridge, let stand for 20 min, elute with 6 mL ethyl acetate. Add the eluate to 1 mL 100 mM sulfuric acid, extract. Add the aqueous layer to 3 mL 2.5 M NaOH, add 25 μL benzoyl chloride, extract with 5 mL ethyl acetate. Wash the ethyl acetate layer with water, evaporate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 50 μL EtOH, 3.5 mL 50 mM pH 8.0 Tris/HCl buffer, and 35 μL esterase (Type 1 porcine liver, Sigma). Heat at 25° for 45 min, add to an activated Sep-Pak C18 SPE cartridge, wash with 5 mL water, elute with 5 mL acetone. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL hexane:EtOH 89:11, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralcel OB + 250 × 4.6 Chiralcel OJ
Mobile phase: n-Hexane:EtOH 89:11
Column temperature: 48
Flow rate: 1.4
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 10 (D), 11.5 (L)
Internal standard: 3-methoxytyramine (54)

OTHER SUBSTANCES
Extracted: metabolites, amphetamine

KEY WORDS
rat; SPE; derivatization; chiral

REFERENCE
Nagai,T.; Kamiyama,S. Simultaneous HPLC analysis of optical isomers of methamphetamine and its metabolites, and stereoselective metabolism of racemic methamphetamine in rat urine, *J.Anal.Toxicol.*, **1991**, *15*, 299–304.

SAMPLE
Matrix: urine
Sample preparation: Adjust pH of 3 mL urine to 11 with 10 M KOH, add to an Extrelut 3 column, let stand for 10 min, elute with 15 mL n-hexane into a tube containing one drop of 3 M HCl. Evaporate the eluate to dryness under a stream of nitrogen at 35°. Add 1.5 mL 8% sodium bicarbonate in water and 1 mL 0.5% sodium naphthoquinone-4-sulfonate in water to the residue, heat at 70° for 20 min, cool, extract with 5 mL carbon tetrachloride. Evaporate the eluate to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Lichrospher 100 RP8
Column: 250 × 4 5 μm Lichrospher 100 RP8
Mobile phase: MeCN:buffer 55:45 (Buffer was 1.361 g KH_2PO_4 in 950 mL, add 1.3 mL methanesulfonic acid, adjust pH to 3 with 5 M KOH, make up to 1 L with water.)
Flow rate: 1
Injection volume: 20
Detector: UV 460

CHROMATOGRAM
Retention time: 7.8
Limit of detection: 60 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine
Noninterfering: acetaminophen, aspirin, amitriptyline, buprenorphine, caffeine, carbamazepine, chlorpromazine, desipramine, dextromethorphan, doxepin, ephedrine, fenfluramine, imipramine, lidocaine, loxapine, meperidine, methadone, methaqualone, naloxone, naltrexone, nicotine, orphenadrine, oxycodone, papaverine, pentazocine, phendimetrazine, phenmetrazine, phentermine, phenylpropanolamine, phenytoin, primidone, procaine, promethazine, propoxyphene, propyphenazone, theobromine, theophylline, trazodone, trifluprom azine, trimethoprim, trimipramine

KEY WORDS
SPE; derivatization

REFERENCE
Ferrara,S.D.; Tedeschi,L.; Frison,G.; Castagna,F. Solid-phase extraction and HPLC-UV confirmation of drugs of abuse in urine, *J.Anal.Toxicol.*, **1992**, *16*, 217–222.

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg Adsorbex SCX cation-exchange SPE cartridge (Merck) with 2 mL MeOH, 1 mL water, and 1 mL 17 mM KH_2PO_4, do not allow to dry. Centrifuge urine at 2000 g for 5 min. 1 mL Urine + 500 μL 50 mM KH_2PO_4, sonicate for 1 min, add to the SPE cartridge, rinse vial with 50 μL 50 mM KH_2PO_4 and add to cartridge, dry cartridge for 1 min, wash with three 500 μL portions of 17 mM KH_2PO_4, wash with 1 mL MeOH, dry under vacuum for 1 min, elute with four 500 μL portions of MeOH:7.3% HCl (97.5:2.5) at a flow rate of 0.5 mL/min, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 3 μm Spherisorb ODS-1
Mobile phase: Gradient. A was water containing 5 mL (8.5 g) 85% orthophosphoric acid and 280 μL (0.22 g) hexylamine per liter. B was MeCN containing 100 mL water, 5 mL (8.5 g) 85% orthophosphoric acid, and 280 μL (0.22 g) hexylamine per liter. A:B 94.5:5.5 for 10.6 min, then to 61:39 over 11 min.
Column temperature: 40
Flow rate: 0.8
Injection volume: 10
Detector: UV 198

CHROMATOGRAM

Retention time: 9
Limit of detection: 30 ng/mL

OTHER SUBSTANCES

Extracted: 3,4-methylenedioxyamphetamine, amphetamine, 4-methoxyamphetamine, phentermine, 3,4-methylenedioxymethamphetamine, 5-methoxy-3,4-methylenedioxyamphetamine, 3,4,5-trimethoxyamphetamine, 3,4-methylenedioxyethylamphetamine, 2,5-dimethoxyamphetamine, 4-bromo-2,5-dimethoxyphenylethylamine, 2,5-dimethoxy-4-methylamphetamine, 4-bromo-2,5-dimethoxyamphetamine, 2,5-dimethoxy-4-ethylamphetamine, mescaline, methoxamine

KEY WORDS

SPE

REFERENCE

Helmlin,H.-J.; Brenneisen,R. Determination of psychotropic phenylalkylamine derivatives in biological matrices by high-performance liquid chromatography with photodiode-array detection, *J.Chromatogr.*, **1992**, *593*, 87–94.

SAMPLE

Matrix: urine
Sample preparation: 5 mL Urine + 4.5 mL MeCN + 500 μL 1 M KOH, centrifuge at 2500 rpm for 10 min, filter (45 μm) the supernatant. Inject on to column A at 180 μL/min 25 μL 50 mM KOH in MeCN:water 20:80, 50 μL filtrate, 25 μL 50 mM KOH in MeCN:water 20:80, and 200 μL MeCN:water 20:80, backflush the contents of column A on to column B with mobile phase, after 18 s remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B. Wash column A with 400 μL MeCN.

HPLC VARIABLES

Column: A 20 × 2 polymeric reagent; B 250 × 4.6 5 μm Supelcosil LC-18-DB (with a guard column) (Prepare polymeric reagent as follows. Prepare a porous rigid resin using a divinylbenzene:ethylstyrene:styrene 24:6:70 mixture with trimethylsilyl modified silica (102 Å average pore size, 1.08 mL/g pore volume, 366 m^2/g surface area, 16-20 μm irregular particle shape, IMPAQ RG 1020 Si silica, PQ Co., Valley Forge PA). Further preparation details are not given but a typical procedure given in the cited reference is as follows. Aerate a mixture of 10 g modified silica in 100 mL water with nitrogen for 15 min, add 10 mL styrene:80% divinylbenzene:t-butyl peroxybenzoate 49:49:2 (remove preservative by passing through a butylcatechol remover (Scientific Polymer, Ontario NY), shake vigorously at room temperature for 4 h, add 150 mL 0.75% polyvinyl alcohol, shake for 4 h, heat at 120° for 24 h while shaking on a Parr instrument, cool to room temperature, filter, wash with 100 mL water, wash with 50 mL MeOH. Add the solid to 500 mL 3 M NaOH in MeOH:water 40:60, shake at room temperature for 14 h (to dissolve the silica), filter, wash with water until the washings are neutral, wash with 100 mL MeOH, dry at 60°. The polymer has similar properties to the template silica (US Pat. 4 933 372 (1990)). Soxhlet extract the resin with dioxane for 8 h (Caution! Dioxane is a carcinogen!). Add 25 g aluminum trichloride in 300 mL dry nitrobenzene to 50 g resin and 100 g 4-chloro-3-nitrobenzoyl chloride, stir mechanically at 60° for 5 h, pour into a mixture of 150 mL DMF, 100 mL concentrated HCl, and 150 g ice, filter. Wash the solid with 300 mL portions of DMF:water 75:25 until the washings are colorless, wash with warm (60°) DMF, wash with six 300 mL portions of dichloromethane:MeOH 2:1. Stir the product in 130 mL 40% benzyltrimethylammonium hydroxide in water, 130 mL water, and 260 mL dioxane at 90° for 8 h, filter, repeat the process. Wash the product with four portions of warm (60°) dioxane. Stir the solid

with 30 mL acetic acid for 15 min, filter. Wash the solid with dioxane until the washings are neutral, wash with six 300 mL portions of dichloromethane:MeOH 2:1 to give a nitrobenzophenol-substituted polymer (J. Org. Chem. 1984, 49, 924). Heat 4 g 9-fluoreneacetic acid, 3.9 mL oxalyl chloride, 30 mL benzene (dried over anhydrous sodium sulfate, Caution! Benzene is a carcinogen!), and 3 drops of triethylamine at 55° for 1 h, evaporate under reduced pressure to remove oxalyl chloride, dissolve the product in 35 mL dichloromethane to give a 120 mg/mL solution of 9-fluoreneacetyl chloride, dilute to obtain a 2 mM solution. Stir 1.3 g nitrobenzophenol-substituted polymer, 4.2 mL 2 mM 9-fluoreneacetyl chloride solution, 300 μL triethylamine, and 20 mL dichloromethane at room temperature for 1 h, filter, wash with three 20 mL portions of MeCN to obtain the reagent, polymer-bound nitrobenzophenol 9-fluoreneacetate (J. Chromatogr. 1992, 609, 103).)

Mobile phase: Gradient. MeCN:water 50:50 for 3.5 min, to 70:30 over 12 min, maintain at 70:30 for 2.5 min, return to initial conditions over 1 min. (Place a 100 × 4.6 30-40 μm silica column before the injector.)

Column temperature: 60 (column A only)

Injection volume: 25-50

Detector: F ex 254 em 305-395

CHROMATOGRAM

Retention time: 13.6

Limit of quantitation: 25 ng/mL

KEY WORDS

derivatization; column-switching

REFERENCE

Bourque,A.J.; Krull,I.S.; Feibush,B. Automated HPLC analyses of drugs of abuse via direct injection of biological fluids followed by simultaneous solid-phase extraction and derivatization with fluorescence detection, *Biomed.Chromatogr.*, **1994**, *8*, 53–62.

SAMPLE

Matrix: urine

Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 500 μL MeOH and 500 μL water. Adjust pH of urine to 10, centrifuge at 1500 g. 2 mL Supernatant + 100 μL 75 μg/mL β-phenylethylamine hydrochloride in water, add to the SPE cartridge, wash with 2.5 mL water, elute with 2 mL MeOH, evaporate the eluate to dryness. Reconstitute in water, add 500 μL 8% sodium bicarbonate, add 500 μL 0.5% 1,2-naphthoquinone-4-sulfonic acid sodium salt, make up to 1.5 mL with water, heat at 70° for 20 min, cool, add an equal volume of chloroform, shake for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and dry it over anhydrous sodium sulfate, filter (0.45 μm), inject a 25 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher Si-60

Mobile phase: EtOH:chloroform:ethyl acetate:n-hexane 1:22:32:45

Flow rate: 2

Injection volume: 25

Detector: UV 280

CHROMATOGRAM

Retention time: 2.6

Internal standard: β-phenylethylamine hydrochloride (4.9)

OTHER SUBSTANCES

Extracted: amphetamine

KEY WORDS

SPE; normal phase; derivatization

REFERENCE

Campins Falcó,P.; Molins Legua,C.; Herráez Hernandez,R.; Sevillano Cabeza,A. Improved amphetamine and methamphetamine determination in urine by normal-phase high-performance liquid chromatography with sodium 1,2-naphthoquinone 4-sulphonate as derivatizing agent and solid-phase extraction for sample clean-up, *J.Chromatogr.B*, **1995**, *663*, 235–245.

SAMPLE
Matrix: urine
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 50 mM pH 11 phosphate buffer. 500 μL Urine + 500 μL 2000 U/mL β-glucuronidase with sulfatase activity (Type H-1, Sigma) in 100 mM pH 5 acetate buffer, heat at 37° overnight, add 500 mg NaCl, add 500 μL 50 mM pH 11 potassium phosphate buffer, adjust pH to 11 with ammonium hydroxide, mix. Add the mixture to the SPE cartridge, wash with 1 mL 50 mM pH 11 potassium phosphate buffer, wash with 1 mL freshly prepared MeOH:water 30:70, wash with 1 mL MeCN, elute with 1 mL freshly prepared MeCN:acetic acid 98:2, elute with 1 mL MeCN:HCl 98:2. Combine the eluates and evaporate them to dryness under a stream of air at room temperature, reconstitute the residue in mobile phase (?), inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: phenyl
Column: Microsorb phenyl
Mobile phase: MeCN:MeOH:50 mM pH 3 potassium phosphate 5:10:85
Flow rate: 1
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 15.6
Internal standard: methamphetamine

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
SPE; rat; methamphetamine in IS

REFERENCE
Law,M.Y.L.; Moody,D.E. Simultaneous quantitation of amphetamine and 4'-hydroxyamphetamine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 2029–2043.

SAMPLE
Matrix: urine
Sample preparation: Condition an Extra-Sep C18 SPE cartridge (Teknokroma) with 1 mL MeOH and 1 mL buffer. Adjust pH of 2 mL urine to ca. 10 with 100 μL concentrated ammonium hydroxide, add 5 μg β-phenylethylamine, add to the SPE cartridge, wash with 5 mL water, wash with 1 mL MeCN, elute with 2 mL MeOH. Add 100 μL EtOH:concentrated HCl 6:1 to the eluate, evaporate to dryness. Reconstitute with 1 mL buffer and 1 mL 0.5% 1,2-naphthoquinone-4-sulfonic acid sodium salt, let stand at room temperature for 10 min, add 2 mL n-hexane:ethyl acetate 50:50, shake for 2 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 500 μL MeCN:water 50:50, inject a 50 μL aliquot. (Buffer was 1% aqueous sodium bicarbonate adjusted to pH 10 with 5 M NaOH.)

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS-C18
Mobile phase: Gradient. MeCN:0.5% propylamine in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 6.3
Internal standard: β-phenylethylamine (4.1)
Limit of detection: 2 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
SPE; derivatization

REFERENCE
Molíns Legua,C.; Campíns Falcó,P.; Sevillano Cabeza,A. Amphetamine and methamphetamine determination in urine by reversed-phase high-performance liquid chromatography with sodium 1,2-naphthoquinone 4-sulfonate as derivatizing agent and solid-phase extraction for sample clean-up, *J.Chromatogr.B*, **1995**, *672*, 81–88.

SAMPLE
Matrix: urine
Sample preparation: 100-300 μL Urine + 100 μL 1.5 M NaOH + 5 μg IS, make up to 1 mL with water, add to an Extrelut 1 SPE cartridge, let stand for 20 min, elute with 6 mL benzene (Caution! Benzene is a carcinogen!). Add the eluate to 1 mL 100 mM sulfuric acid, extract. Remove the aqueous layer and add it to 3 mL 1.5 M NaOH, add 5 μL benzoyl chloride, vortex vigorously, extract twice with 3 mL portions of n-hexane. Combine the organic layers and wash them twice with 3 mL portions of water, evaporate the organic to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Chiralcel OB-H
Mobile phase: n-Hexane:isopropanol 90:10
Column temperature: 55
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 6.5 (D), 10 (L)
Internal standard: l-p-methoxyamphetamine (12)
Limit of detection: 30 ng

OTHER SUBSTANCES
Extracted: amphetamine, ethylamphetamine

KEY WORDS
rat; derivatization; SPE; chiral

REFERENCE
Nagai,T.; Kamiyama,S.; Matsushima,K. Analysis of time-lapse changes of d- and l-enantiomers of racemic ethylamphetamine and stereoselective metabolism in rat urine by HPLC determination, *J.Anal.Toxicol.*, **1995**, *19*, 225–228.

SAMPLE
Matrix: urine
Sample preparation: Dilute urine 20-fold or more. 500 μL Diluted urine + 50 μL 500 ng/mL (+)-2,5-dimethoxyamphetamine hydrochloride + 100 μL 500 mM NaOH + 2 mL benzene (Caution! Benzene is a carcinogen!), shake for 15 min, centrifuge at 1200 g for 5 min. Remove 1.8 mL of the organic phase and add it to 220 μL 50 mM HCl, shake for 15 min, centrifuge at 1200 g for 5 min. Remove 200 μL of the aqueous phase and add it to 40 μL 250 mM NaOH, add 50 μL 330 mM pH 7.8 phosphate buffer, add 250 μL MeCN, add 25 μL 3 mM (-)-1-(9-fluorenyl)ethyl chloroformate, let stand at room temperature for 24 h, add 30 μL 100 mM glycine in water, let stand for 30 min, add 750 μL pentane, vortex for 2 min, centrifuge at 1200 g for 5 min. Remove the organic layer and evaporate it to dryness in a centrifugal evaporator at room temperature, reconstitute the residue in 300 μL MeCN:water 50:50, inject a 100 μL aliuot.

HPLC VARIABLES
Column: 150 × 4.6 3 μm Adsorbosphere HS C18

Mobile phase: MeCN:THF:20 mM pH 3.6 sodium acetate buffer 25:21:54
Flow rate: 1
Injection volume: 100
Detector: F ex 265 em 330

CHROMATOGRAM
Retention time: 42 (L), 44 (D)
Internal standard: (+)-2,5-dimethoxyamphetamine (33)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
rat; derivatization; chiral

REFERENCE
Sukbuntherng,J.; Hutchaleelaha,A.; Chow,H.-H.; Mayersohn,M. Separation and quantitation of the enantiomers of methamphetamine and its metabolites in urine by HPLC: Precolumn derivatization and fluorescence detection, *J.Anal.Toxicol.*, **1995**, *19*, 139–147.

SAMPLE
Matrix: urine
Sample preparation: Condition a 200 mg Bond Elut C18 SPE cartridge with 1 mL MeOH and 1 mL 1% pH 10 sodium bicarbonate buffer. 2 mL Urine + 400 μL 8% pH 10 sodium bicarbonate buffer, mix, centrifuge at 1500 g for 2 min, add a 2 mL aliquot of the supernatant to the SPE cartridge, wash with 3 mL water, pass 500 μL 2% sodium 1,2-naphthoquinone 4-sulfonate through the cartridge, pass 500 μL 1% pH 10 sodium bicarbonate buffer through the cartridge, let stand at room temperature for 15 min, wash with 3 mL water, elute with 1 mL MeCN:water 50:50, inject a 20 μL aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil ODS
Mobile phase: Gradient. MeCN:buffer from 40:60 to 50:50 over 2.5 min, to 70:30 over 0.5 min, maintain at 70:30 for 1.5 min, to 85:15 over 1 min, maintain at 85:15 for 1.5 min. (Buffer was 5 mL/L propylamine in water.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 5.6
Internal standard: β-phenylethylamine (3.6)
Limit of detection: 400 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
derivatization; SPE

REFERENCE
Campíns-Falcó,P.; Sevillano-Cabeza,A.; Molíns-Legua,C.; Kohlmann,M. Amphetamine and methamphetamine determination in urine by reversed-phase high-performance liquid chromatography with simultaneous sample clean-up and derivatization with 1,2-naphthoquinone 4-sulphonate on solid-phase cartridges, *J.Chromatogr.B*, **1996**, *687*, 239–246.

SAMPLE
Matrix: urine
Sample preparation: Inject 15 μL urine, inject a mixture of 5 μL 20 mM 9-fluorenylmethyl chloroformate in MeCN and 45 μL water, and inject 10 μL buffer on to column A and elute to

waste with mobile phase A. After 2.8 min backflush the contents of column A on to column B with mobile phase B and start the gradient, monitor the effluent from column B. At the end of the run condition column A with 1 mL mobile phase A. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm LiChrospher 100 PR-C18
Mobile phase: A water; B Gradient. MeCN:water from 40:60 to 70:30 over 15 min. to 100:0 over 5 min.
Flow rate: A 0.35; B 1
Injection volume: 15
Detector: F ex 264 em 313

CHROMATOGRAM
Retention time: 17.8
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine, ephedrine, norephedrine, 3-phenylpropylamine, pseudoephedrine

KEY WORDS
column-switching; derivatization; on-column derivatization

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. Determination of amphetamine and related compounds in urine using on-line derivatization in octadecyl silica columns with 9-fluorenylmethyl chloroformate and liquid chromatography, *J.Chromatogr.B*, **1996**, *679*, 69–78.

SAMPLE
Matrix: urine
Sample preparation: Inject 50 μL urine on to column A and elute to waste with mobile phase A, after 2 min inject a mixture of 25 μL 0.5% sodium 1,2-naphthoquinone-4-sulfonate in water and 25 μL buffer on to column A, stop the flow of mobile phase A, after 10 min start pump A, after 5 min backflush the contents of column A on to column B with mobile phase B and start the gradient, monitor the effluent from column B. After each run flush column A with ethyl acetate for 1 min, n-hexane for 1 min, and ethyl acetate for 1 min, re-equilibrate with mobile phase A. (Buffer was 4% sodium bicarbonate adjusted to pH 10 with 10% NaOH.)

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 5 μm Hypersil ODS C18
Mobile phase: A water; B Gradient. MeCN:0.5% propylamine hydrochloride in water from 40:60 to 50:50 over 2.5 min, to 70:30 over 1 min, maintain at 70:30 for 4.5 min.
Flow rate: 1
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 7
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: amphetamine

KEY WORDS
column-switching; derivatization; on-column derivatization

REFERENCE
Herráez-Hernández,R.; Campíns-Falcó,P.; Sevillano-Cabeza,A. On-line derivatization into precolumns for the determination of drugs by liquid chromatography and column switching: Determination of amphetamines in urine, *Anal.Chem.*, **1996**, *68*, 734–739.

SAMPLE
Matrix: urine
Sample preparation: Condition a Bond Elut SCX SPE cartridge with 10 mL MeOH:aqueous ammonia 98:2, 10 mL MeOH, and 30 mL water. 10 mL Urine + 10 mL 50 mM pH 7.8 potassium phosphate buffer, mix, add to the SPE cartridge, wash with 2 mL water, wash with 10 mL MeOH, elute with 3 mL MeOH:2% aqueous ammonia 98:2. Add 30 μL glacial acetic acid and 30 μL 1 mg/mL N-ethylaniline in MeOH to the eluate, inject a 5-50 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 ULTRON ES-PhCD β-cyclodextrin phenylcarbamate-bonded silica (Shinwa Chemical Industries, Kyoto)
Mobile phase: MeCN:MeOH:buffer 10:30:60 (Buffer was 50 mM pH 6.0 potassium phosphate for UV detection or 100 mM pH 6.0 ammonium acetate for MS detection.)
Column temperature: 25
Flow rate: 1
Injection volume: 5-50
Detector: UV 220, MS, Shimadzu LCMS-QP1100EX, thermospray, positive ion mode, filament off, vaporizer 230°, ion source 300°, m/z 150

CHROMATOGRAM
Retention time: 12 (D, UV), 15 (L, UV), 15 (D, MS), 17.5 (L, MS)
Internal standard: N-ethylaniline (17, UV only)
Limit of detection: 50 ng/mL (D, UV detection), 100 ng/mL (L, UV detection), 10 ng/mL (D, scan MS), 20 ng/mL (L, scan MS), 0.8 ng/mL (D, SIM MS), 1.0 ng/mL (L, SIM MS)

OTHER SUBSTANCES
Extracted: metabolites, amphetamine, p-hydroxymethamphetamine

KEY WORDS
SPE; chiral

REFERENCE
Katagi,M.; Nishioka,H.; Nakajima,K.; Tsuchihashi,H.; Fujima,H.; Wada,H.; Nakamura,K.; Makino,K. Direct high-performance liquid chromatographic and high-performance liquid chromatographic-thermospray-mass spectrometric determination of enantiomers of methamphetamine and its main metabolites amphetamine and p-hydroxymethamphetamine in human urine, *J.Chromatogr.B*, **1996**, *676*, 35–43.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 20 μL 100 μM 1-phenylethylamine in water + 400 μL concentrated HCl, heat at 80° for 1 h, cool, neutralize with 600 μL 25% ammonia, add 5 mL 10% sodium carbonate solution, add 2 mL 500 mM pH 10.5 sodium borate buffer, add 2 mL chloroform:isopropanol 75:25, vortex for 1 min, centrifuge at 12.5° at 1500 g for 10 min, repeat the extraction. Combine the organic layers and remove a 100 μL aliquot, add 10 μL acetic acid to the aliquot, evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 50 μL carbonate buffer, add 50 μL 10 mM fluorescein-4-isothiocyanate in EtOH, mix, heat in the dark at 80° for 15 min, inject a 20 μL aliquot. (Prepare carbonate buffer by adjusting the pH of 200 mM sodium bicarbonate to 9.0 with 200 mM sodium carbonate.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Daisopak SP-120-5-ODS (Daiso, Osaka)
Mobile phase: Gradient. MeCN:20 mM pH 7.9 sodium phosphate buffer 20:80 for 16 min then 24:76 (step-gradient).
Flow rate: 0.8
Injection volume: 20
Detector: F ex 496 em 518

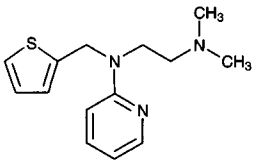
CHROMATOGRAM
Retention time: 35.2
Internal standard: 1-phenylethylamine (26.6)
Limit of detection: 5.5 nM

OTHER SUBSTANCES
Extracted: metabolites, amphetamine, norepinephrine

KEY WORDS
derivatization

REFERENCE
Al-Dirbashi,O.; Kuroda,N.; Akiyama,S.; Nakashima,K. High-performance liquid chromatography of methamphetamine and its related compounds in human urine following derivatization with fluorescein isothiocyanate, *J.Chromatogr.B*, **1997**, *695*, 251–258.

Methapyrilene



Molecular formula: $C_{14}H_{19}N_3S$
Molecular weight: 261.39
CAS Registry No.: 91-80-5, 33032-12-1 (fumarate), 135-23-9 (HCl)
Merck Index: 6027
Lednicer No.: 1 54

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone,

phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

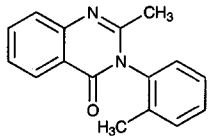
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenyl-

butazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Methaqualone



Molecular formula: $C_{16}H_{14}N_2O$
Molecular weight: 250.30
CAS Registry No.: 72-44-6, 340-56-7 (HCl)
Merck Index: 6028
Lednicer No.: 1 353

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 µL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 µL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 µm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 226

CHROMATOGRAM

Retention time: 4.19
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine;

prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

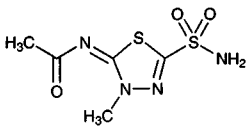
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, me-

phesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Methazolamide



Molecular formula: $C_5H_6N_4O_3S_2$
Molecular weight: 236.28
CAS Registry No.: 554-57-4
Merck Index: 6031
Lednicer No.: 1 250

SAMPLE

Matrix: blood, urine
Sample preparation: To 500 μL whole blood, plasma, or urine add 20 μL 1 mg/mL acetazolamide solution and 2.5 mL 50% ammonium sulfamate, vortex for 30 s. (Place the tube containing whole blood in boiling water for 30 s and then quickly in cold water.) Add 5 mL ethyl acetate, vortex, centrifuge at 3000 g for 10 min, transfer the organic layer to 5 mL pH 8.0 phosphate buffer, vortex, centrifuge at 3000 g for 10 min, transfer the organic layer to 500 μL pH 10.0 glycine buffer, vortex for 30 s, centrifuge at 3000 g for 5 min. Aspirate and discard the organic layer, add 500 μL ether to the remaining glycine buffer layer, vortex for 1 min, discard the ether phase. Vent the aqueous layer for about 30 min and inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Altima C18 (Alltech)
Mobile phase: MeCN:100 mM pH 4.0 sodium acetate 20:80
Flow rate: 1
Injection volume: 20
Detector: UV 285

CHROMATOGRAM

Retention time: 7.18
Internal standard: acetazolamide (4.55)

KEY WORDS

plasma; whole blood